CHAPTER 5

MARINE BIOTOXIN MANAGEMENT AND CONTINGENCY PLAN

Introduction to Harmful Algal Blooms (HABs) in Mississippi Waters

The Gulf Coast of Mississippi consists of approximately 85 miles of sand shores interspersed by three major bays (Bay St. Louis, Biloxi Bay and Pascagoula Bay) and numerous bayous consisting of approximately 72,000 acres of coastal wetlands. The Pearl, Tchoutacabouffa, Biloxi, Escatawpa and Pascagoula Rivers are the primary freshwater inputs from the mainland. These nutrient-rich outflows influence the productivity of the relatively eutrophic, shallow waters of the MS Sound between the Gulf Island National Seashore barrier island chain (Cat, Ship, Horn, and Petit Bois Islands in Mississippi and Dauphin Island in Alabama) and coastal mainland. Further influence from the Mobile Bay and Atchafalya-Mississippi River basin plumes greatly contribute to the nutrient and sedimentation levels of the Mississippi Sound. For these reasons, phytoplankton blooms in Mississippi coastal waters are highly diverse and primarily dominated by euryhaline species, with occasional high concentrations (500,000 to >1,000,000 cells/l) of chlorophytes and cryptophytes originating from freshwater sources during sustained and seasonal rain events.

Historically, there have been three *Karenia brevis* blooms in Mississippi waters. These blooms occurred in 1996, 2007, and 2015. Currents, winds, and storm events can push *K. brevis* from its normal high-salinity, oligotrophic environment off the mid to southern Florida coast northwest into Mississippi waters. Sampling done by the Gulf Coast Geospatial Center (GCGC) after Hurricane Katrina showed the presence of *K. brevis* in numbers exceeding 50,000 cells/L during the month of October from stations to the north and south of Horn Island. During September and October of 2005, public reports and inquiries by fisherman were received on the Gulf Coast Research Laboratory website, reporting, and asking for explanations of unusually high fish kills and respiratory problems while fishing on or near the north shores of Horn Island. These public reports alluded to a potential prolonged *K. brevis* HAB event following Hurricane Katrina and temporally corresponded to reports of bloom events on the Florida panhandle and in Mobile Bay, Alabama. Infrastructure damage due to the hurricane prevented any official actions on these data.

As in nearby Louisiana and Alabama waters, reports of *Pseudo-nitzschia* species in near shore water samples are common during winter and spring months. These counts have exceeded 80,000 cells/L in Mississippi waters. This diatom is responsible for production of domoic acid, but this toxin can be at low levels despite high population counts. Thus far, high toxic levels have not been reported in Mississippi samples, but the ability of this organism to thrive in these waters makes it a potential threat.

1. Marine Fisheries, Shellfish Bureau

The MDMR Shellfish Bureau conducts phytoplankton sampling twice per month during oyster season. Samples are collected at two locations on oyster reefs in the western MS Sound and two locations on oyster reefs in Biloxi Bay. These locations correspond to the northernmost and southernmost perimeter of productive oyster reefs off the shores of Pass Christian and East Biloxi. In addition, there is one sample location within the oyster aquaculture growing waters south of Deer Island. When an influx of freshwater is released into the western MS Sound, a fifth sampling location is added. This additional site is located within the St. Joe oyster reef southwest of Bayou Caddy near the Louisiana/Mississippi State line.

Bimonthly samples are collected using a 20 μm mesh plankton net and enumerated for the presence of all phytoplankton. Shellfish staff follows the sampling protocols set forth by the NOAA Phytoplankton Monitoring Network (PMN). All results are recorded in the MDMR data collection program and are reported to the PMN. Environmental and water quality data are collected during each sample trip. This includes air and water temperature, salinity, dissolved oxygen, pH, turbidity, wind speed and direction. This data is collected using a water quality meter, secchi disk, and Kestrel. Sample qualitative analysis is conducted in the MDMR Marine Fisheries Lab using a phase contrast microscope. In the event of a bloom, quantitative analysis is conducted using an inverted microscope to determine the number of cells per liter.

In addition, MDMR personnel conduct field observations for water discoloration during monthly routine water sampling trips. If an area is suspected of a toxic bloom, samples are collected and analyzed immediately. MDMR personnel investigate possible toxic blooms reported by credible sources, primarily: adjacent state agencies, federal agencies, local health agencies, and academic institutions.

2. Marine Fisheries, Shrimp and Crab Bureau

Because there is no evidence that brevetoxins from *Karenia brevis* build up in the tissue of penaeid shrimp species (*Farfantepenaeus aztecus*, *Farfantepenaeus duorarum*, and *Litopenaeus setiferus*) and blue crab (*Callinectes sapidus)*, the MDMR Shrimp and Crab Bureau will not close the shrimp and blue crab fisheries; however, the general public should be advised not to consume any of the internal organs (i.e. gills, hepatopancreas,

intestines, etc.), eggs, or any dead or dying shrimp and/or crabs in the event of a *K. brevis* fish kill.

There is no research to support any type of closure for species of penaeid shrimp and blue crabs in any of the gulf states. Landsber, et.al (2009) described a benthic mortality of penaeid shrimp species and mole crabs (*Emerita spp.*) preceding the September 2005 *Karenia brevis* event at Boca Chica Beach, Texas by two weeks. The direct correlation of the red tide, toxicosis, and indicators of poor water quality was suspected, but not confirmed (Landsber, et.al 2009). MDMR Shrimp and Crab Bureau will continue to research for any articles relating to *Karenia brevis* associated to penaeid shrimp species and blue crabs.

History of HAB's in Mississippi Waters

In March of 2007, MDMR developed a Marine Biotoxin Contingency Plan that defines HAB monitoring efforts for marine and estuarine shellfish growing areas. The contingency plan was revised and updated in 2012, 2016, and 2019.

The MDMR Shellfish Bureau began routine phytoplankton sampling of Area 5 in Biloxi Bay. This area opened to the harvest of oyster during the 2016-17 oyster season for the first time in over 20 years. It became necessary to monitor for HABS once harvesters began removing oysters from this area for public consumption.

1. Summer 2020 – HAB Bloom of *Alexandrium spp.*, *Cochlodinium spp.*, *Prorocentrum spp.*, and *Nitzschia spp.*

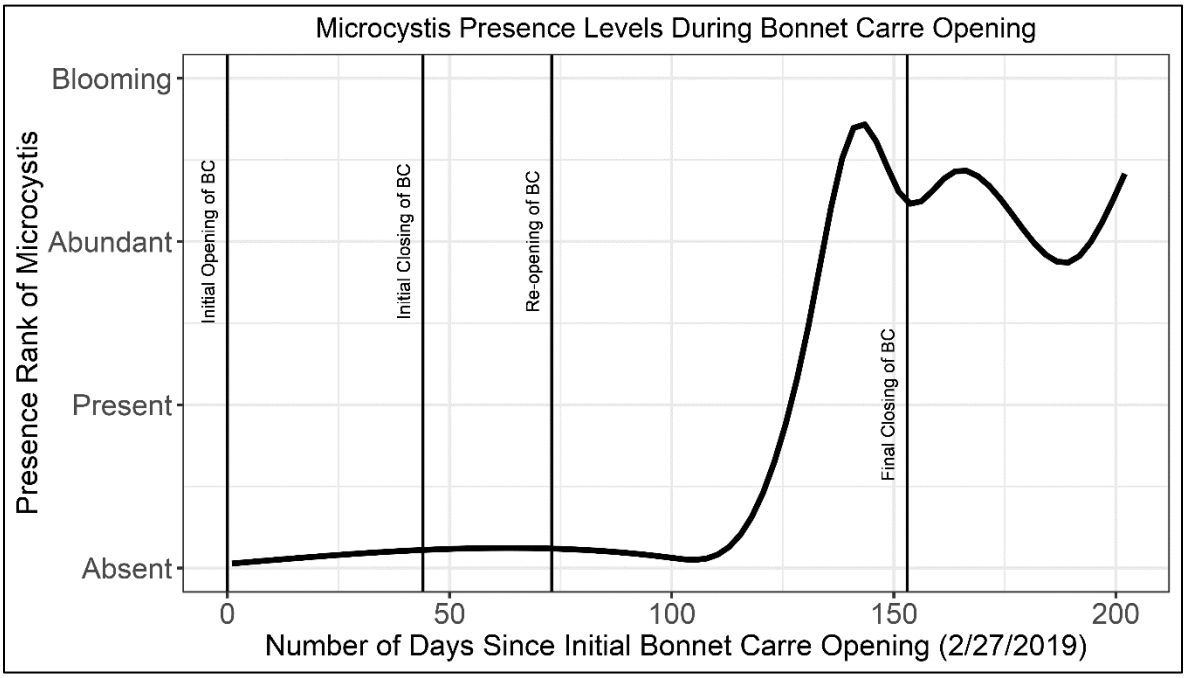
From July 13, 2020, to July 23, 2020, a HAB of *Alexandrium spp., Cochlodinium spp., Prorocentrum spp., and Nitzschia spp.* persisted on the south side of Deer Island in Approved Area 5C which houses the state aquaculture park. *Alexandrium spp.* produces several toxins, specifically a Paralytic Shellfish Poisoning (PSP) causing the toxin, saxitoxin. *Cochlodinium spp.*, renamed *Margalefidinium spp.*, is known as a marine fish killing microalgae and can also be a toxin producer of hemolytic and neurotoxic-like substances causing fish and oyster mortality. *Prorocentrum spp.* is a producer of okadaic acid known to cause Diarrhetic Shellfish Poisoning (DSP). *Nitzschia spp.* produces domic acid which causes Amnesic Shellfish Poisoning (ASP). Over the course of the bloom, a total of 22 whole water samples were collected to monitor the HAB activity. Both seawater samples and oyster meat samples were sent off for toxin analysis which included: saxitoxins, domic acid, okadaic acid and dinophysistoxins. The toxin results for the seawater samples and tissue samples were nondetectable above their respective method detection limits.

2. Summer, Fall 2019 – Cyanobacteria bloom of *Microcystis spp*.

A HAB of *Microcystis spp*. persisted along the MS Gulf Coast from June to October 2019. *Microcystis spp*. is a freshwater cyanobacteria with a long history of wreaking havoc on freshwater systems across the US. They cause HABs when nutrients such as nitrogen and phosphorus are abundant in the environment. *Microcystis spp*. is a hardy plankton that can survive in a wide variety of environmental conditions and has impressive adaptability to environmental change. The historical presence of *Microcystis spp.* within the MS Sound is unknown. There are no known documented cases of its presence until 2019. MDMR has been monitoring phytoplankton populations among oyster reefs since 2009.

The influx of freshwater from the openings of the Bonnet Carré Spillway carried high levels of nutrients, such as nitrogen and phosphorus, into the MS Sound. MDMR staff began increased monitoring of phytoplankton population and density on February 27, 2019. The first siting of *Microcystis spp*. within the MS Sound was on June 12, 2019. During the months of June through August, in addition to *Microcystis spp.*, there were multiple blooms of different plankton including: *Akashiwo spp.; Dolichospermum spp. (*formerly *Anabaena spp.); Scrippsiella spp.;* and *Chattonella spp.* These blooms were mostly short-lived unlike *Microcystis spp.* which persisted.

There were 1,333 water samples collected between MDMR and MDEQ to monitor HAB activity. Quantitative analysis was conducted for the presence of HABs. MDMR had toxin analysis conducted on 92 seafood tissue samples and 77 water samples. MDMR worked with the Dauphin Island Sea Laboratory (DISL) to conduct enzyme-linked immunosorbent assay (ELISA) testing and Green Water Laboratory to conduct LC-MS/MS (liquid chromatography, mass spectrometry) testing for toxin presence.



3. Winter 2015-16 – *Karenia brevis*

K. brevis caused a widespread HAB of red tide across the MS Sound. This HAB moved from east and south into the sound at the beginning of December 2015 and persisted within the sound for approximately 37 days. During this time, MDMR personnel collected 263 samples for analysis of algal presence and cell counts. This includes 165 cell counts and 98 plankton net tows. The Mississippi oyster reefs were closed to the harvest of oysters on December 11, 2015 and remained closed until January 26, 2016.

On November 10, 2015 the MDMR received word from the Alabama Department of Public Health (ADPH) that a *K. brevis* bloom was present along the Alabama coastline. At this point, MDMR personnel began water sampling along the MS Gulf Coast in anticipation of the bloom moving into state waters. Samples were initially collected using a plankton net and following the standards of the NOAA PMN program for collection (i.e., three-minute tow, etc.). The first siting of *K. brevis* in Mississippi waters occurred on December 1, 2015. On December 10, MDMR personnel started collecting liter surface grab water samples, preserving these samples in Lugol's iodine solution and conducting in-house cell counts of *K. brevis*. The last positive count of *K. brevis* in Mississippi waters was on January 6, 2016.

On January 13, 2016 MDMR personnel collected seven oyster meat samples from various productive oyster reefs in the western MS Sound. These samples were processed and sent to the DISL for ELISA testing to pre-screen samples for the presence or absence of

brevetoxins. Oyster batches from each area sampled were determined to contain brevetoxin-3 equivalent levels well below the FDA guidance level of 800 parts per billion.

On January 18, 2016, MDMR personnel collected 6 meat samples for analysis of brevetoxin presence by mouse bioassay. These samples were initially sent to the Florida Fish and Wildlife Conservation Commission, Florida Wildlife Research Institute (FWRI) Laboratory in St. Petersburg, Florida where the samples were processed for further analysis. The biotoxin laboratory at FWRI is the official state laboratory of Florida for testing algal toxins in shellfish following harvesting bans caused by red tide. At this time, the FWRI lab was back logged on analyzing samples due to the widespread impact of this bloom. Alternatively, the FWRI sent Mississippi samples to their sister company, Resource Access International in Brunswick, Maine, where the mouse bioassay was conducted. The results of the mouse bioassay were received by email on January 25, 2016. All the mice survived the bioassay test, i.e. all tests were less than 20 mouse units (MU) per 100 grams of oyster meat.

On January 26, 2016, the western Mississippi sound oyster reefs were declared free of brevetoxins and able to reopen for the harvest of oysters: however, the reefs remained closed due to other environmental complications (i.e., rainfall total, Pearl River stage exceeding management plan criteria and the Bonnet Carré spillway opening in Louisiana).

4. 2007 - *Karenia brevis*

It has been suggested that there was a *K. brevis* bloom that entered waters of the MS Sound however no sampling was conducted by the MDMR. These suggestions were made by the NOAA Marine Lab at Stennis which conducts satellite imagery. In 2007, a bloom was present on the satellite imagery.

5. Fall 1997 - *Karenia brevis*

Mississippi experienced a bloom of *K. brevis* which was reported as *Gymnodinium breve* at this time. Over a four-month period, numerous water samples and oyster meat samples were collected. Meat samples were tested using mouse bioassay. This event forced the closure of several oyster harvesting reefs in the western MS Sound.

6. Minor HAB events

There have been phytoplankton blooms across the MS Sound investigated over the past several years, none of which caused a shellfish reef closure. These include:

2014 – *Scrippsiella spp*. bloom

2013 – *Ceratium furca* bloom

2011 – *Chatonella subsalsa* bloom

2010 – *Ceratium furca* bloom

Rules and Regulations for HAB events

The harvest of shellfish contaminated by the presence of marine biotoxin-producing organisms in numbers sufficient to cause a public health risk will be prevented by the closure of affected waters. Identification of the dinoflagellate *K. brevis* within the water column, exceeding 5,000 cells per liter will immediately trigger a closure of affected shellfish growing areas in Mississippi. Reefs that are affected will remain closed to harvesting until concentrations drop below 5,000 cells per liter. Contaminated shellfish that have been harvested from an affected area shall be returned to the waters upon the presence of marine biotoxin-producing organisms in numbers sufficient to cause a health risk.

If unknown biotoxin-producing organisms are encountered, technical assistance from the Food and Drug Administration (FDA) and others will be sought in determining closing and re-opening criteria and procedures.

The MDMR will immediately disseminate information on the occurrences of HABS and toxicity in shellfish meats to the shellfish industry, local health agencies, and adjacent states by the most appropriate and effective communication means available.

MDMR's authority to close waters or embargo shellfish is described in *Mississippi Code of 1972, §§ 49-15-3, 49-15-15*, *49-15-21, 49-15-36, and 49-15-44.*

HAB Response Team Qualifications

On January 1, 2019, the MDMR Marine Fisheries Office established the HAB Response Team. The goal of this task force is to respond to any potential harmful algal bloom that occurs within the MS Sound, report details of the response to management and quickly identify potential public health risks associated with HABs. HAB Response Team members are required to participate in the following training.

- Attend a 7-day training workshop titled *Gulf of Mexico Harmful Algal Bloom Taxonomy Training Workshop* currently held at Bigelow University in Boothsbay, Maine.
- Participate in 20 hours of MDMR lab exercises where the following knowledge is tested: identification of current algae present in samples taken within the MS Sound; identification of the most threatening HAB's; identification of freshwater HAB's that may affect MS waters during the Bonnet Carré spillway opening.
- Participate in a training session on the reporting of algal samples to NOAA's Phytoplankton Monitoring Network.

Equipment and Supplies

1. Sampling using the net-tow process.

- Net with 20um mesh
- Bottles
- Stopwatch
- Bottle carrying case
- Equipment used to measure environmental parameters (YSI, Kestrel, Secchi disc)
- Data sheet

2. Sampling using the surface grab process.

- 1 Liter glass jar
- Bottle carrying case
- Equipment used to measure environmental parameters (YSI, Kestrel, Secchi disc)
- Data sheet
- Lugol preservative (only if the samples will not be analyzed by the lab within 24 hours of collection)

Phytoplankton Sampling Protocol

The MDMR Fisheries Bureau collects monthly phytoplankton samples for observation of the presence of phytoplankton from 15 sites across the MS Sound. Samples are collected by boat throughout the season from indicator stations, to be assayed for the presence of HABS. In addition, samples will be collected when shellfish growing areas are open to harvest of oysters or prior to opening. The MDMR will coordinate with the Mississippi Department of Environmental Quality (MDEQ) South Regional Office, USM Gulf Coast Research Laboratory (GCRL), and Alabama Department of Public Health (ADPH), or other appropriate agencies for assessment of samples when necessary. Figure 1 below is a map of the routine sampling locations. These locations correspond to the northernmost and southernmost perimeter of productive oyster reefs off the shore of Pass Christian and the western and southernmost perimeters of the Biloxi Bay Reef.

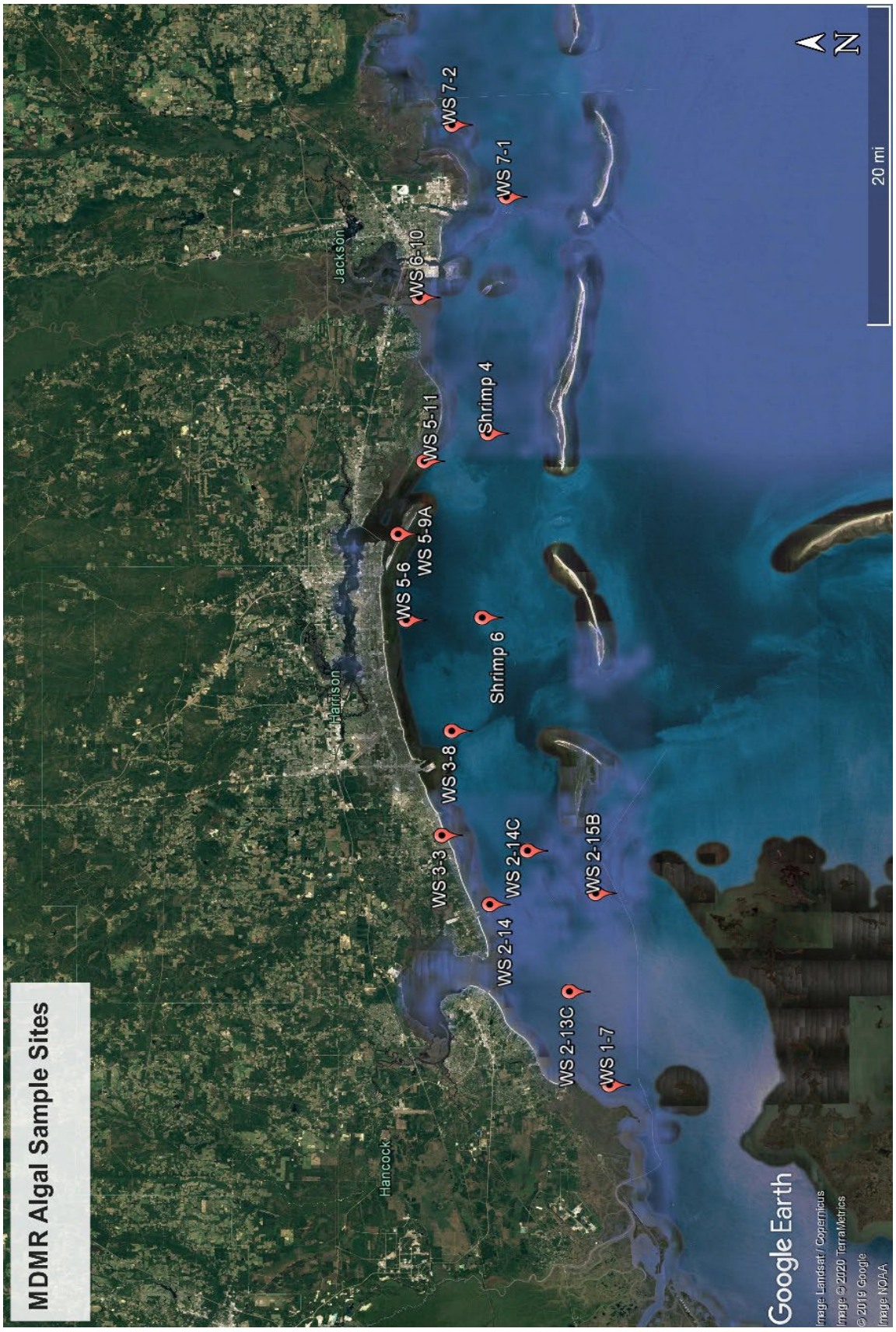


Figure 1: Routine monthly Algal Sampling Sites

When an influx of freshwater is released into the western MS Sound, usually from the opening of the Bonnet Carré Spillway, a third sampling location is added. This additional site is located within the St. Joe oyster reef southwest of Bayou Caddy. A 'Bonnet Carré Opening' report will be completed for each year that this spillway opens. Refer to these reports for additional information.

Shellfish Staff follow the procedures of the NOAA PMN for sampling protocol. All results are reported to PMN and recorded in the MDMR in house data collection spreadsheet. Environmental water quality data is collected during each sample trip. This includes air and water temperature, salinity, dissolved oxygen, pH, and turbidity. Sample analysis is conducted with a phase contrast microscope. Qualitative analysis is recorded however, in the event of a bloom, quantitative analysis will be conducted to determine possible toxicity levels.

MDMR personnel make field observations by boat during routine, monthly water sampling trips of shellfish growing areas. Areas of discolored water suspected to be from a bloom of phytoplankton will be noted, GPS coordinates recorded, and a water sample collected when possible. In the event of a fish kill or other type of threat indication, MDMR personnel will investigate phytoplankton blooms reported by a reliable source.

Reports to the MDMR of a marine biotoxin detected in the adjacent waters of bordering states will intensify monitoring efforts. Over flights of Mississippi's shellfish growing areas may be conducted as deemed necessary. Flight paths will include as many active reef areas as possible. MDMR personnel will investigate indications of a potential bloom by collecting water samples for analysis were deemed appropriate. MDMR, GCRL, and other appropriate personnel will make identifications and cell counts per liter where appropriate. When possible, the MDMR will coordinate control actions taken by other state or federal agencies involved.

Phytoplankton samples may be conducted periodically in addition to opportunistic samples (i.e., fish kills, visible blooms, etc.)

3. Whole water / Surface Collection

- Collect sample below the surface of the water by inverting the 1-liter jar, then sticking your arm below the surface of the water up to your elbow (approximately 1 ft below the surface), then turning the jar over for water collection.
- Collect sample as close to the bow of the boat as possible.

4. Net Tow Collection

- Using a plankton net and a bottle, drop the net into the water next to the vessel.

- Remove all air in the bottle by dipping the net into the water so that the bottle is full. Then fully dip the net ensuring that the bottle bottom is not floating.
- Drop the net again and begin pull the net alongside the vessel. Make sure the mouth of the net is completely submerged under the surface of the water as you begin pulling.
- Drive boat in low gear in a circle during the tow ensuring you stay close to the coordinates for that sample.
- Pull for exactly 3 minutes. Use a stopwatch or timer to be exact.
- Once 3 minutes has lapsed, pull the net next to the boat and allow the water to drain from the net on its own.
- Grab the net at the top and close the net in your fist. With your other hand, hold the sample bottle attached to the net. Gently swish the water back and forth down the sides of the net to collect phytoplankton. Try to keep as much water as possible. The goal here is to wash the cells into the cup from the net sides.
- Remove the bottle from net and secure with a loosely placed lid.

5. Environmental Data Collection

During routine phytoplankton sampling, it is important to collect as much information as possible including, but not limited to:

- Site Name
- Date and Time of sample collection
- GPS coordinates of sample location
- Air quality
- Air temperature
- Wind Speed and Direction
- Water quality information from surface and bottom including:
- Water Temperature
- Dissolved oxygen
- Salinity
- pH
- Turbidity
- Record observation of surrounding area (discolored water, dead fish, tide line, large boats near, any activity such as dredging, etc.)

6. Preserving a Sample with Lugol

Lugol is the preservative used to fix phytoplankton cells. Not all cell bodies will be preserved with Lugol. For a 1-liter jar, use 7mL of Lugol. The sample only needs to be

preserved if the lab staff are unable to examine the sample within 24 hours of collection. If using Lugol, pour the Lugol into the sample after collection, secure the lid and gently tilt the bottle back and forth twice to ensure rapid fixation of phytoplankton cells. Lugol can be added any time within 8 hours of sample collection.

7. Transporting a Phytoplankton Sample

Loosely secure the lid onto the jar. Do not tighten the lid. Allow some air flow during transportation. Store the sample in a shaded area as direct sunlight will damage the cells. Do not store the sample on ice as freezing will destroy the cells. Deliver the sample to the lab and inform the HAB response team that samples have been delivered. It is imperative that the sampler contacts one of the HAB task force members in the morning before collecting a sample. Place the samples in the wet lab so it is visible to staff. Be sure to leave all associated paperwork with the sample bottles including environmental data collection.

HAB Task Force Members	Title	Phone
Kristina Broussard	Biological Program Coordinator – Shellfish Bureau	228-234-8001
Alicia Carron	Marine Scientist – Shellfish Bureau	228-523-4088
Megan Fleming	Marine Scientist – Finfish Bureau	228-523-4167
Tiffany Weidner	Biological Program Coordinator – Shrimp and Crab Bureau	228-523-4019

8. Data Management

Data collected during any HAB investigation is stored on the MDMR M drive. The following parameters are used when identifying cell count abundance. This graph was reproduced from the Florida Fish and Wildlife Commission.

Description	*Karenia brevis* abundance	Possible effects (*Karenia brevis* only)
NOT PRESENT-BACKGROUND	0 - 1,000 cells/L	no effects anticipated
VERY LOW	> 1,000 - 10,000 cells/L	possible respiratory irritation; shellfish harvesting closures when cell abundance equals or exceeds 5,000 cells/L
LOW	> 10,000 - 100,000 cells/L	respiratory irritation; shellfish harvesting closures; possible fish kills; probable detection of chlorophyll by satellites at upper range of cell abundance
MEDIUM	> 100,000 - 1,000,000 cells/L	respiratory irritation; shellfish harvesting closures; probable fish kills; detection of surface chlorophyll by satellites
HIGH	> 1,000,000 cells/L	as above, plus water discoloration

Coordinates of Routine Sample Stations

Monthly sampling during oyster season:

Site Name	Site Coordinates
2-14	30.284950, -89.237117
2-37 (Formerly 2-15B)	30.196433, -89.225267
5-9A	30.361850, -88.850067
5-15	30.375317, -88.830617
5-16	30.390767, -88.855367

Monthly Sampling:

Site Name	Site Coordinates
1-7	30.184233, -89.425617
2-32 (Formerly 2-13C)	30.218100, -89.328000

2-14	30.284950, -89.237117
2-35 (Formerly 2-14C)	30.253533, -89.180850
2-37 (Formerly 2-15B)	30.196433, -89.225267
3-3	30.325167, -89.165017
3-8	30.31705, -89.055917
5-6	30.35515, -88.939967
5-9A	30.361850, -88.850067
5-11	30.340050, -88.774100
6-10	30.343900, -88.602667
7-1	30.270717, -88.497450
7-2	30.31595, -88.421683
Shrimp 4	30.28667, -88.745
Shrimp 6	30.29167, -88.9375